



Gastrointestinal Issues in Autism Spectrum Disorder

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While autism spectrum disorder (ASD) is characterized by communication impairments, social abnormalities, and stereotypic behaviors, several medical comorbidities are observed in autistic individuals. Of these, gastrointestinal (GI) abnormalities are of particular interest given their reported prevalence and correlation with the severity of core autism-related behavioral abnormalities. This review discusses the GI pathologies seen in ASD individuals and the association of particular GI conditions with known genetic and environmental risk factors for autism. It further addresses how GI abnormalities can affect the neuropathological and behavioral features of ASD, as well as the development of autism-related endophenotypes such as immune dysregulation, hyperserotonemia, and metabolic dysfunction. Finally, it presents emerging evidence for a gut-brain connection in autism, wherein GI dysfunction may contribute to the pathogenesis or severity of ASD symptoms.

Keywords: gastroenterology, gut, immunity, intestine, neurodevelopment

Autism spectrum disorder (ASD) is a serious neurodevelopmental condition that is diagnosed based on the presence and severity of core behavioral symptoms—deficient social interactions, impaired communication, and the presence of repetitive behavior or restricted interests. In addition to the spectrum of symptoms seen within these principal diagnostic criteria, ASD individuals display a wide range of neurological comorbidities, including intellectual disability, epilepsy, and anxiety and mood disorders, as well as non-neurological comorbidities, including blood hyperserotonemia, immune dysregulation, and GI abnormalities.^{1,2} The striking clinical heterogeneity across individuals that share the same overall ASD diagnosis is consistent with the prevailing notion that the disorder has multiple underlying causes and developmental manifestations.

Several genetic and environmental susceptibility factors have been identified that increase the risk for features of autism, but few cases of ASD can be attributed to a defined etiology. Moreover, molecular diagnostics are not available for the reproducible identification of ASD; as yet, the disorder is diagnosed based on standardized behavioral assessments. Without a clear understanding of the mechanistic basis of ASD, therapeutics for treating the core symptoms of autism are limited; most drugs prescribed to ASD individuals are used to treat autism-related conditions, such as anxiety, hyperactivity, epilepsy, and obsessive-compulsive behaviors. Collectively, the heterogeneity of ASD and the

associated challenges in identifying specific causes, treatments, and molecular biomarkers for the disorder point to the need to better define the clinical subtypes of ASD and to tailor research studies to well-delineated subclasses of ASD individuals.

GASTROINTESTINAL PROBLEMS IN AUTISM

Of the many medical comorbidities associated with ASD, GI distress has gained significant attention because of its reported prevalence and association with symptom severity. In fact, 7 out of the 11 children that Leo Kanner³ described in his landmark article defining “infantile autism” are described as having eating/feeding or dietary problems, supporting an early association of ASD with GI issues. Of the GI problems reported in subsets of autistic individuals, the most common are chronic constipation, diarrhea, and abdominal pain^{4–6} (see Figure 1). Gastroesophageal reflux, bloody stools, vomiting, and gaseousness are also elevated in some autistic individuals, as are signs of GI inflammation, such as lymphoid nodular hyperplasia, complement activation, and elevated pro-inflammatory cytokines, and intestinal pathologies, such as enterocolitis, gastritis, and esophagitis.^{4,6} Increased intestinal permeability is linked to autism and hypothesized to have detrimental effects not only on intestinal barrier integrity^{7,8} but also on the systemic metabolome, with potential for translocation of intestinal metabolites or bacteria and consequent immune activation.⁹ Furthermore, food allergies, altered dietary nutrient intake, and metabolic disruptions have been associated with ASD.^{10–12} Autistic individuals with comorbid GI abnormalities exhibit altered carbohydrate digestion.¹³ Taken together, the variety of GI conditions, dietary issues, and enteric immune abnormalities reported in ASD individuals suggests that GI dysfunction can contribute to the manifestation of core symptoms of autism.

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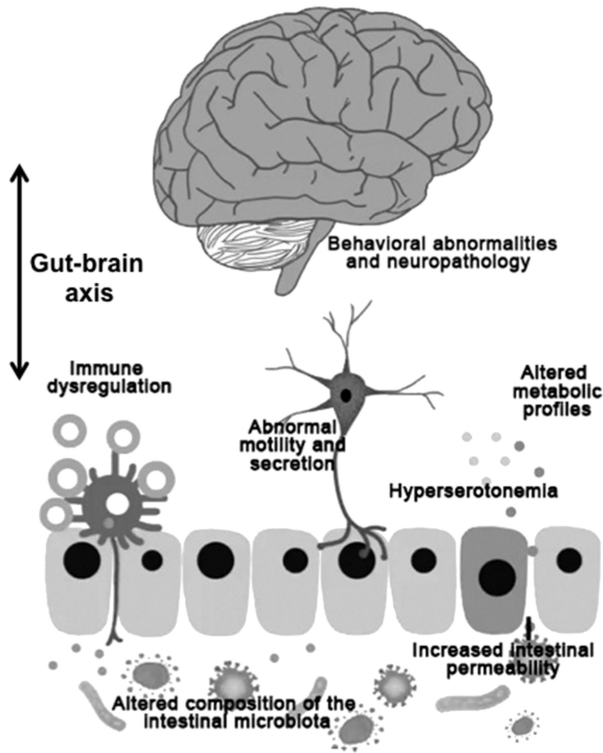


Figure 1. Gastrointestinal influences on symptoms of autism spectrum disorder. Gastrointestinal abnormalities, such as increased intestinal permeability, altered composition of the intestinal microbiota, and dysregulated gastrointestinal motility and secretion, are described in subsets of individuals with ASD. Such phenotypes can affect the development of other autism-associated phenotypes. For example, gastrointestinal disruptions can influence the production or metabolism of serotonin from gut enterochromaffin cells, the major producers of peripheral serotonin, leading to the well-replicated molecular association of hyperserotonemia with ASD. Also, increased intestinal permeability can lead to the leakage of bacterially derived or bacterially modulated metabolites across the intestinal epithelial and into the bloodstream, which may underlie the elevated levels of bacterial metabolites and alterations in the urinary and serum metabolome seen in autistic individuals. Furthermore, gastrointestinal issues can lead to widespread immune dysregulation, as is observed in human autism. Overall, changes in the gastrointestinal tract can influence higher-order behavior and brain function via the gut-brain axis, driven by direct connections of the intestinal epithelium to the brain stem and secondary projection sites via the vagus nerve, and by indirect connections of the gut to the brain via alterations in immunity and metabolism.

Several studies report that autistic individuals exhibit dysbiosis, or altered composition, of the intestinal microbiota.^{13–19} Though the specific species of microbes altered in ASD versus controls has varied from one study to the next, three studies employing different methodologies report that autistic individuals display significantly elevated levels of *Clostridium* species.^{18,20,21} In a study of autistic individuals with comorbid GI symptoms compared to typically developing controls with GI symptoms, pyrosequencing of ileal and cecal biopsies revealed a specific association of *Sutterella* species and the presence of anti-*Sutterella* serum antibodies in those with autism.²² Increasing evidence indicates that changes in the composition of the commensal microbiome can alter complex behaviors, including anxiety-like behavior,²³ emotional or depressive behavior,²⁴ and locomotor activity,²³

among others.^{25–27} Whether microbiota changes can contribute to the development or progression of autism symptoms is unknown.²⁸ Significantly increased levels of the *Clostridium*-derived metabolite para-cresol,²⁹ along with various other bacterially modulated metabolites,^{11,29–32} is detected in the urine of autistic children, suggesting a possible interaction between gut dysbiosis, intestinal permeability, and metabolic dysfunction in autism. Further studies utilizing gnotobiotic animals will provide insight into the potential role of autism-associated microbiome changes on GI function and behavioral phenotype.

Despite the many studies on GI dysfunction in ASD, whether GI issues are more common in the autism population versus controls is unclear. The reported prevalence of GI abnormalities in individuals with ASD ranges from 9% to 91% across different studies, and similarly broad ranges are seen specifically for the incidence of particular GI symptoms, including constipation, diarrhea, and abdominal discomfort in ASD children.^{30,33–38} Many of these studies have significant methodological limitations, such as inappropriate experimental controls, overwhelming sample heterogeneity, inadequate sample size, selection and referral bias, and retrospective study design.^{4,6} In addition, each study varies in the method of assessing and defining GI symptoms, which is likely to contribute to the widely variable reported frequency of GI problems in autistic individuals versus controls. Nonetheless, a systematic review of relevant medical literature published in 2009 or earlier led an expert panel of ASD and GI research scientists and clinicians to assert that “despite the limitations in type and quality of available evidence, the preponderance of data were consistent with the likelihood of a high prevalence of gastrointestinal symptoms and disorders associated with ASDs.”⁴ Consistent with this conclusion, a recent multicenter study of over 14,000 ASD individuals reports a higher prevalence of inflammatory bowel disease (0.83% versus 0.54%) and other bowel disorders (11.74% versus 4.5%) in ASD patients compared to hospitalized controls.² Interestingly, parental reports of GI impairment in their autistic children correspond well to clinical diagnoses of GI abnormalities, corroborating the reliability of studies using parental accounts to demonstrate elevated incidence of GI complications in autistic individuals versus controls.³⁹ Further prospective, population-based studies are needed to determine with confidence whether GI complications are more prevalent in particular subsets of ASD individuals.

POTENTIAL GASTROINTESTINAL MANIFESTATIONS OF GENETIC AND ENVIRONMENTAL RISK FACTORS FOR AUTISM

The molecular causes of ASD are largely unknown but are believed to originate from a combination of genetic and environmental risk factors. Several single-gene polymorphisms, including those affecting *CNTNAP2*, *SHANK3*, and *NLGN3/4*, have been found to increase the risk for

autism.⁴⁰ In addition, a number of copy-number variations and chromosomal abnormalities, such as the 15q11-q13 duplication and 16p11.2 deletions or duplications, have been associated with autism. Given the high heritability of ASD and the elevated concordance of autism in monozygotic versus dizygotic twins,⁴¹ much research has focused on genetic contributions to the disorder. However, the finding that dizygotic twins share much higher rates of autism concordance compared to non-twin siblings indicates a role for shared environmental risk factors in elevating autism risk.^{41,42} Specifically, a recent study reports an estimated 55% of autism risk driven by environmental insults and 37% of that risk attributable to genetic susceptibility.⁴² Environmental risk factors known to increase the risk for symptoms of autism include maternal use of the teratogens thalidomide or valproic acid and maternal infection during pregnancy.⁴³ It is hypothesized that environmental risk factors can also contribute to the heritability of autism via the epigenetic programming of autism-associated dysfunction. Along these lines, a recent whole-genome epigenetic study of ASD twins and controls reports a number of DNA methylation sites in ASD individuals that correlate with symptom severity,¹² suggesting a role for environmental risk factors in inducing genetically heritable disease susceptibility.

Increasing evidence suggests that GI complications can arise as a result of genetic and environmental risk factors for ASD. One susceptibility gene that is particularly interesting in this regard is *c-Met*, a proto-oncogene that encodes MET receptor tyrosine kinase. This membrane receptor mediates the effects of its specific ligand, hepatocyte growth factor, on a variety of biological processes, including cellular division, angiogenesis, immune function, intestinal epithelial development, and brain development. Notably, the *c-Met* promoter variant rs1858830 reflects a common single nucleotide polymorphism that increases the risk for ASD and is distinctively associated with ASD in individuals with co-occurring GI dysfunction.⁴⁴ Consistent with this finding, hepatocyte growth factor levels are decreased in serums from autistic children with comorbid GI disease when compared to non-autistic children with GI disease, autistic children without GI disease, and healthy controls (non-autistic children without GI disease).⁴⁵ This result strongly suggests that MET hypofunction due to the ASD-associated common genetic variant rs1858830 specifically predisposes a subset of ASD individuals that exhibit GI problems. In addition to a potential role for MET as a risk factor for autism-associated GI symptoms, MET protein expression is decreased in the temporal cortex of postmortem brains from autistic individuals compared to controls,⁴⁶ suggesting a direct effect of MET hypofunction on brain function and development. Whether MET effects on autism-associated GI abnormalities and behavior arise through parallel, but independent pathways, is unknown. It will be interesting to determine and contrast the effect that selective MET

hypofunction in the GI tract has on brain development and function, versus the effect that selective MET hypofunction in the brain has on GI physiology.

Another susceptibility gene for ASD that may be linked to GI dysfunction is *SLC6A4*, which encodes the integral membrane transporter for the neurotransmitter serotonin (SERT).^{47–49} Several rare SERT coding variants have been identified as risk factors for ASD, all of which result in hyperfunctional serotonin transporter activity.^{48,50} Such SERT overactivity on circulating platelets can cause hyperserotonemia,⁴⁹ or increased blood serotonin, which is the most well-replicated biomarker for ASD, seen in an estimated 30% of afflicted individuals.^{51,52} Consistent with this pathophysiology, transgenic mice expressing a human ASD-associated SERT variant exhibit the hyperserotonemia phenotype, along with core autism-related behavioral abnormalities and altered serotonergic neurotransmission.⁵³ Notably, since endocrine cells of the GI tract known to produce over 90% of the body's serotonin and intestinal enterocytes are known to express SERT,⁵⁴ ASD-associated SERT polymorphisms are likely to disrupt GI serotonin metabolism. Such effects would alter serotonin-related processes in the GI tract, such as the regulation of mucosal immune responses and the activation of enteric reflexes that underlie gut motility, secretion, and sensation. Alterations in GI levels of serotonin and serotonergic signaling are associated with a variety of pathologic conditions, including irritable bowel syndrome, inflammatory bowel disease, and idiopathic constipation.^{55,56} Thus, specific genetic variations that predispose for ASD can elicit molecular alterations that lead to ASD-associated GI abnormalities (see Figure 2).

Several environmental risk factors have been found to increase the risk for ASD, and a number of these also display interesting links to ASD-related GI abnormalities. Of particular note is maternal immune activation,⁵⁷ which is regarded as a principal non-genetic cause of autism—based on several large epidemiological studies linking various maternal infections, elevated pro-inflammatory markers, and immune-related insults to increased autism risk in the offspring.^{58–60} Moreover, several studies have revealed altered immune profiles and function in not only the peripheral immune organs, but also the brains and GI tracts, of children and adults with ASD.^{61,62} Given that the immune compartments of the GI tract harbor an estimated 80% of all immune cells in the human body, it is likely that immune activation and immune dysregulation can lead to GI dysfunction. Inflammatory cytokines and chemokines are known to regulate intestinal epithelial development and integrity in addition to modulating the activity of enteric neurons, which control GI motility. Interestingly, modeling maternal immune activation in mice sufficiently yields chronic peripheral and enteric immune dysregulation, deficient intestinal barrier integrity, ASD-related behavioral abnormalities, and hallmark ASD neuropathologies, thus recapitulating neural, immune, and GI endophenotypes

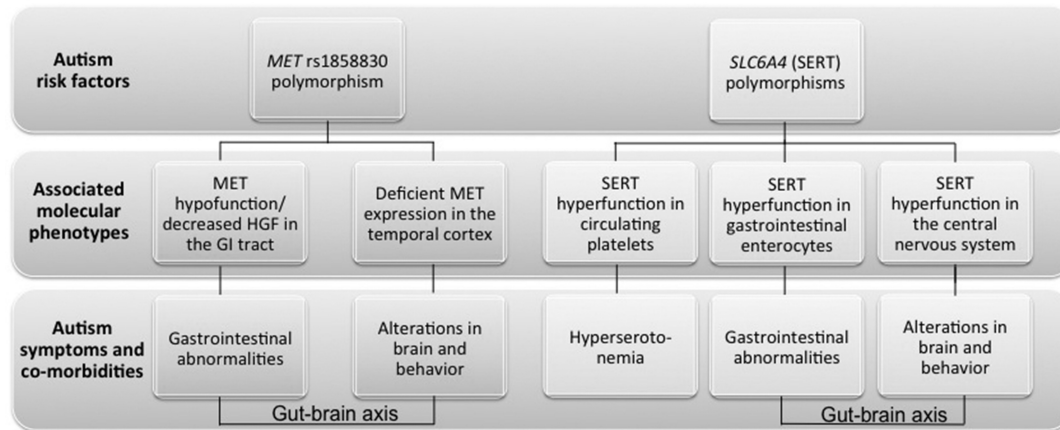


Figure 2. Links between genetic risk factors for autism and gastrointestinal abnormalities. A number of environmental and genetic risk factors for autism exhibit compelling associations with gastrointestinal dysfunction. For example, the autism-associated variant of *c-MET*, encoding the MET tyrosine kinase, is enriched particularly in autistic individuals with comorbid gastrointestinal dysfunction. Consistent with this finding, decreases in *MET* expression are observed in temporal cortex of postmortem autism brains, and decreases in the endogenous ligand of MET, called hepatocyte growth factor (HGF), are seen in the gastrointestinal tracts of autistic individuals. Similarly, the autism-associated polymorphisms in *SLC6A4*, encoding the serotonin transporter (SERT), can lead to SERT hyperfunction not only in the brain but also in the gastrointestinal tract, resulting in abnormalities in behavior and gastrointestinal function, as well as autism-related increases in blood serotonin levels. That gastrointestinal abnormalities can influence brain and behavior is supported by studies of the gut-brain axis and also by findings that gastrointestinal complications correlate with the severity of behavioral abnormalities seen in individuals with autism.

observed in human autism.⁶³ Whether such environmental risk factors as maternal infection are epidemiologically associated particularly with human ASD and co-occurring GI conditions or postnatal immunological dysfunction is unknown and warrants further research.

Another intriguing risk factor for ASD with connections to GI abnormalities and genetic risk is maternal autoreactive antibody production. Several groups have identified immunoglobulins from plasma of mothers with ASD children that display abnormal reactivity against fetal antigens, including GAD65 in cerebellar Purkinje cells⁶⁴ and myelin basic protein.⁶⁵ Although many of these “autoantibodies” are still detected, albeit at a lower frequency, in typically developing, non-ASD controls, one particular subtype of reactive maternal autoantibodies has been detected specifically in autism cases and not in controls.⁶⁵ Remarkably, these ASD-specific maternal autoantibodies are strongly associated with the functional *c-Met* C allele that confers genetic susceptibility to ASD with comorbid GI abnormalities.⁶⁶ The mechanistic pathways underlying this link between genetic risk and autoantibody production is unclear but can be mediated by immune or GI factors. Overall, the strong associations between GI dysfunction, immune dysregulation, and associated genetic and environmental susceptibility factors form a promising avenue for future research into possible convergent pathways accounting for ASD core behavioral symptoms and associated comorbidities (see Figure 1).

THE GUT-BRAIN CONNECTION IN AUTISM

The notion that autism-associated GI abnormalities can contribute to the manifestation of core ASD symptoms is compelling in light of a rich literature on the gut-brain-axis,^{67,68}

where the GI tract and brain communicate bidirectionally to regulate a variety of functional processes (see Figure 1). Several direct and indirect mechanisms potentially mediate how molecular alterations in the GI tract affect brain development and function. Changes in the GI microenvironment can alter the activation of vagal nerve afferents,^{26,27} which contact the lining of the intestinal epithelium and extend directly to the brain stem, specifically innervating cells of the nucleus tract solitarius. Neurons in the nucleus tract solitarius further send outputs to a number of secondary projection sites, including the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. This mechanism governs the communication of mechanosensory and chemosensory information from the visceral tissues to the brain, regulating such GI processes as motility, secretion, and satiety. Notably, vagal nerve activation also modulates a number of complex behaviors, including emotional and cognitive behavior, and anxiety or stress.

GI effects on the immune system can also influence brain and behavior. Functional GI disorders are strongly linked to GI inflammation, intestinal permeability, and altered composition of the gut microbiota. Various immunological abnormalities have been observed in the GI tracts of autistic individuals, including leukocyte infiltration, complement activation, lymphoid hyperplasia, and pro-inflammatory cytokine responses.^{4,69} Consistent with these findings, autistic individuals with comorbid GI problems are reported to exhibit altered immune responses and transcriptional profiles compared to autistic controls without GI symptoms.^{70–72} Several immune abnormalities are also observed systemically in autistic individuals, including differential leukocyte responses to stimulation, altered T lymphocyte abundance, and abnormal levels of immunoglobulin and cytokines.⁶²

Interestingly, alterations in peripheral immune profiles correlate with the severity of behavioral scores of autistic individuals. In particular, changes in the levels of particular plasma cytokines, such as TGF β , p-selectin, and MIF, are associated with the severity of communication deficits, stereotypy, and hyperactivity in the Autism Diagnostic Interview–Revised, Autism Diagnostic Observation Schedule, or Aberrant Behavior Checklist assessments.⁷³ In addition, altered plasma immunoglobulin profiles correlate with the severity of symptoms as indicated by Aberrant Behavior Checklist scores.⁷⁴ Altogether, these findings, along with a vast literature on immunological effects on synaptic pruning, neural transmission, neurogenesis, and glial differentiation, among other neurological processes,⁷⁵ suggest that changes in immunological status can affect autism symptoms.

Whether GI abnormalities in autistic individuals actually contribute to the development, persistence, or intensity of core autism symptoms is unknown. It is plausible that GI issues that result in distress or discomfort can potentiate problem behaviors, including self-injurious and stereotypic vocal or motor behaviors. For example, abdominal discomfort in an autistic individual may be related to presentations of abnormal mouthing or posturing behaviors, self-injury to the abdomen or other areas to detract from GI pain, or vocal groaning or screaming.⁵ In addition, other comorbidities, including sleep disturbance and abnormal feeding behaviors such as pica, can arise as a result of GI complications, including pain and acid reflux.⁷⁶ In addition, some symptoms of autism may develop as a result of both neural and GI dysfunction, whereby the inability of an autistic individual to communicate GI discomfort or distress forms the underlying basis for abnormal clinical presentations.

It is interesting to consider the possibility that primary neural dysfunction in autism can cause downstream problems in the GI tract. Several psychiatric illnesses, such as depression and anxiety disorder, are linked to GI problems.^{77,78} In fact, it is estimated that 60% of patients suffering from functional GI disorders exhibit comorbid major affective disorders.⁷⁹ Supporting the ability of the brain to modulate gut function, animal studies demonstrate that chronic psychosocial stress leads to immune and GI abnormalities, including alterations in the intestinal microbiome.^{80–83} Similarly, in a mouse model of depression, intracerebroventricular exposure to the catecholamine-depleting agent reserpine not only induces depression-related behavior but also predisposes animals to acute experimental colitis.⁸⁴ Perhaps most well-known of these paradigms is the association of chronic stress with both depression and gastric ulcers.

Whether GI complications in autism arise as a result of primary neural dysfunction or through a parallel independent pathway is unclear. However, that the intensity of particular GI abnormalities is reported to correlate with the severity of core autism behavioral symptoms suggests that GI problems can contribute to the manifestation of

ASD-related behaviors.^{85,86} Autistic children with comorbid GI symptoms display more severe irritability, anxiety, and social withdrawal, among other behavioral problems,^{39,87} supporting a role for the gut-brain connection in ASD. In addition, many reports provide compelling evidence that limited diet or antibiotic treatment can effectively ameliorate autism-related behavioral problems. For example, casein and gluten-free diets are commonly used by autistic individuals and reported to improve behavior.^{88–90} Casein and gluten exclusion is hypothesized to affect brain function by preventing undigested casein and gluten peptides from accessing the bloodstream, crossing the blood-brain barrier, and initiating neurotoxic responses via binding to brain opioid receptors. Exclusion is also hypothesized to elicit beneficial effects on intestinal lesions by preventing immunological responses against casein and gluten in the GI mucosa. Notably, however, the utility of the casein- and gluten-free diet to treat core symptoms of autism is largely derived from anecdotal accounts and lacks empirical support from well-designed scientific studies. Antibiotic treatment is also reported to improve autism symptoms. In a study of 11 children with regressive-onset autism, oral vancomycin treatment reduced autism-associated behavioral abnormalities,⁹¹ supporting a role for dysbiosis of the gut microbiota in the development or persistence of autism symptoms. Interestingly, vancomycin treatment provided only short-term improvement of autism-related behavioral abnormalities; the beneficial effects of vancomycin were lost after stopping antibiotic treatment, suggesting an active and persistent interaction between microbial colonization and behavioral outcomes. Whether dietary or probiotic treatments can be used to relieve GI symptoms, ameliorate immune dysregulation, and improve behavioral symptoms in autistic individuals should be further evaluated in large, well-controlled studies.

CONCLUSIONS AND PERSPECTIVES

ASD is tremendously heterogeneous not only in the presence and severity of its diagnostic behavioral features, but also in the presence and severity of a wide range of medical comorbidities. The challenges faced in identifying the underlying causes, molecular biomarkers, and specific treatments for ASD warrant the need to study well-defined subclasses of autistic individuals. A preponderance of evidence suggests that a significant subset of autistic individuals exhibit GI abnormalities and that GI issues can contribute to the clinical manifestations of ASD-associated symptoms, including abnormal behavior, immune dysregulation, and metabolic dysfunction (see Figure 1). Additional prospective, population-based studies are needed to determine the frequencies of specific GI symptoms in autism and to support existing evidence that GI conditions are enriched in particular subsets of ASD individuals. Further studies into the impact of genetic and environmental risk factors for ASD on the development and function of the GI tract will provide greater insight into a molecular basis

for medical comorbidities seen in autistic individuals (see Figure 2). Moreover, examining how GI disturbances affect brain and behavior in animal models for autism can reveal promising targets for the development of biomolecular diagnostics and therapeutics for ASD. Finally, in view of the widespread use of exclusion diets, probiotics, and antibiotic treatments by autistic individuals, a greater understanding of the role of the intestinal microbiota on immunity, metabolism, and behavior is needed to facilitate the establishment of proper guidelines for autism treatment, and to promote the development of novel and tractable therapeutics for ASD.

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